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Dietary restriction of rodents decreases aging rate without affecting initial mortality rate – a meta-analysis

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Summary

Dietary restriction (DR) extends lifespan in multiple species from various taxa. This effect can arise via two distinct but not mutually exclusive ways: a change in aging rate and/or vulnerability to the aging process (i.e. initial mortality rate). When DR affects vulnerability, this lowers mortality instantly, whereas a change in aging rate will gradually lower mortality risk over time. Unraveling how DR extends lifespan is of interest because it may guide toward understanding the mechanism(s) mediating lifespan extension and also has practical implications for the application of DR. We reanalyzed published survival data from 82 pairs of survival curves from DR experiments in rats and mice by fitting Gompertz and also Gompertz–Makeham models. The addition of the Makeham parameter has been reported to improve the estimation of Gompertz parameters. Both models separate initial mortality rate (vulnerability) from an age-dependent increase in mortality (aging rate). We subjected the obtained Gompertz parameters to a meta-analysis. We find that DR reduced aging rate without affecting vulnerability. The latter contrasts with the conclusion of a recent analysis of a largely overlapping data set, and we show how the earlier finding is due to a statistical artifact. Our analysis indicates that the biology underlying the life-extending effect of DR in rodents likely involves attenuated accumulation of damage, which contrasts with the acute effect of DR on mortality reported for *Drosophila*. Moreover, our findings show that the often-reported correlation between aging rate and vulnerability does not constrain changing aging rate without affecting vulnerability simultaneously. Key words: caloric restriction; Gompertz; Gompertz–Makeham; mice; rats; senescence.

Introduction

Restriction of food intake is one of the few treatments that reliably extends life across taxa, ranging from yeast, to fruit flies, to rodents (Mair & Dillin, 2008; Fontana *et al.*, 2010; Nakagawa *et al.*, 2012), with contradictory reports in rhesus monkeys (Colman *et al.*, 2009; Mattison *et al.*, 2012). This experimental intervention has consequently attracted a considerable research effort to unravel the mechanisms underlying the lifespan extension, and studies suggest among other things the involvement of IGF-1, redox, TOR, and

sirtuin signaling pathways, reviewed in Weindruch *et al.* (2008), Masoro (2009), and Speakman & Mitchell (2011).

However, a basic property of how dietary restriction (DR) extends lifespan demographically is still unresolved, especially in mammals (Masoro, 2006). When organisms live longer, this can either be due to a reduction in vulnerability to the aging process (i.e. initial mortality rate, frailty, delaying the onset of senescence) or a slower rate of aging (Pletcher *et al.*, 2000), or a combination of these. Identifying the means by which a life-extending effect is achieved is of interest because both ways have fundamentally different predictions for the biological processes involved (Partridge *et al.*, 2005). Aging rate is the result of a cumulative process, for example somatic damage, which is at least partly irreversible. Physiological ability to tolerate this damage comprises vulnerability to the aging process. Different levels of vulnerability can thus result in different rates of mortality with the same amount of incurred damage.

In which way lifespan is extended is also crucial in optimizing a treatment in terms of life extension, because it affects the return from a period of treatment in terms of lifespan gain (Partridge *et al.*, 2005). When vulnerability to the aging process is lowered, starting DR at any time during an individual's lifespan, if this individual is still alive, can allow one to reap the full benefits of DR (Vaupel *et al.*, 2003). In contrast, when the rate of aging is affected, the duration and timing of DR treatment will determine the individual's lifespan gain.

Indeed when DR is applied for a longer time, the life-extending effect is larger in rodents (Merry, 2002). Note, however, that this demographic effect can arise due to either a longer period of lower vulnerability to mortality or a longer period of a lowered rate of aging. Decreases in age-related, for example De Cabo *et al.* (2004), or disease markers, for example Lane *et al.* (2000), can also arise via both mechanisms. Increased interest in this issue was sparked when DR in *Drosophila* was shown to alter vulnerability rather than aging rate, in controlled diet switching experiments (Good & Tatar, 2001; Mair *et al.*, 2003). This suggests that dietary manipulations can change mortality rate almost immediately and that this effect is reversible (Good & Tatar, 2001; Mair *et al.*, 2003). It may also suggest that vulnerability to the aging process and aging rate can be affected independently. This contrasts with the repeated observation that they are often found to be correlated, which led to the formulation of the compensation law of mortality in the reliability theory of aging (Gavrilov & Gavrilova, 2001). In short, the reliability theory of aging states that organisms are composed of redundant units, which fail at a similar rate, and when redundancy is depleted, the organism dies. Mortality rates converge because at the end of the life of organisms, redundancy is depleted and mortality rate thus converges to the failure rate of these units. Failure rate is hypothesized to be relatively invariable within a species (Gavrilov & Gavrilova, 2001), causing a correlation between vulnerability and aging rate.

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To test how DR in rodents affects vulnerability vs. aging rate, we collected published survival data and reanalyzed them by fitting Gompertz and also Gompertz–Makeham models. We subsequently subjected the resulting parameter estimates of both vulnerability and aging rate to a meta-analysis, and in this context, we also tested whether the DR effect is modulated by moderator variables such as species, and degree and timing of DR. An earlier study that summarized DR experiments concluded that in rodents, DR decreased both vulnerability and aging rate (Nakagawa *et al.*, 2012). Here, we show that this earlier finding was due to a statistical artifact. Instead, we conclude from our reanalysis of published full survival trajectories of DR experiments in rats and mice that DR slows aging without affecting vulnerability to the aging process.

Results

We found 50 papers that fitted our inclusion criteria. These studies contained 82 pairs of survival curves, with a total of 8624 individuals. Both Gompertz and Gompertz–Makeham models (Eqn 1 and 2) were fitted with maximum-likelihood estimation (MLE) (Pletcher *et al.*, 2000), in which a corresponds to vulnerability to the aging process (i.e. initial mortality rate) and b to the rate of aging. In the Gompertz–Makeham model, the Makeham parameter, m , represents a risk that is equal at all ages.

The overall effect sizes per model showed that aging rate (b) was affected by DR, whereas the vulnerability to the aging process (a) and Makeham (m) parameter were not (Table 1, Figs 1 and 2, S1 and S2). The nonsignificant effect of DR was to lower a , but the life-extending effect gained via a was markedly lower than the gain via the decreased aging rate, b (Table 1).

We included the following moderators in a meta-analysis (Table S1) of each of the five parameters we obtained: species (rat or mouse); sex; age at treatment start, ln (days); whether DR was applied gradually; the level of DR (% of control); and whether dietary or caloric (i.e. the diet was manipulated so that limiting nutrients were supplied in a similar amount) restriction was applied. In the models for each parameter including all moderators together, only age at treatment start (ln) turned out significant for b in both

the Gompertz and Gompertz–Makeham models (estimate: 0.14, 0.13; $P = 0.004$, $P = 0.006$; respectively). Also when we tested the moderators separately for each estimated parameter, we only detected that starting at an older age decreased the effect DR had on b (similar slopes as in full models; all other $P > 0.07$). For all parameters, there was significant residual heterogeneity ($Q > 128$, $P < 0.001$), except for the a in the Gompertz–Makeham model ($Q = 90$, $P = 0.22$), indicating that there is scope for other unknown moderating variables. There were no indications for publication bias in any of the parameters (regression tests, $P > 0.05$ and funnel plot inspection).

The results we obtained differ from an earlier extensive analysis on this topic, which used different statistical methodology, linear regression of hazard ratios against age (Nakagawa *et al.*, 2012). It has previously been shown that when mortality parameters are estimated using linear regression, instead of maximum-likelihood or in part nonlinear survival fitting, this causes a substantial bias that is most pronounced at the intercept, corresponding to a (Mueller *et al.*, 1995; Pletcher, 1999; Promislow *et al.*, 1999; Yen *et al.*, 2008). Using simulated data (see Experimental procedures), we find that when we vary b without varying a , the method employed by Nakagawa *et al.* results in a substantial bias with a striking quantitative fit to the effect on the intercept they reported (Dialog S1A). We also re-analyzed the present data set using the method of Nakagawa *et al.* and compared the results with the results obtained using our methodology, MLE, which also revealed a biased estimate of the intercept (Dialog S1B). In addition, we re-analyzed the set of papers included in Nakagawa *et al.* with MLE and find similar results as for the present data set, DR affects b with a small nonsignificant effect on a (Dialog S1C).

Discussion

Our re-analysis of survival trajectories of DR experiments in rodents shows that DR slows the rate of aging without affecting the vulnerability to the aging process (Fig. 1, Table 1). Our analysis thus suggests that aging rate can be lowered without increasing vulnerability (Fig. 2), contrary to the compensation law of mortality (Gavrilov & Gavrilova, 2001). This suggests that aging rate is not a species-specific set property, but a property that can be modified. The difference of our conclusions with a recent analysis of DR literature by Nakagawa *et al.* emphasizes that statistical methodology used to fit survival trajectories can lead to false conclusions.

The data we used were obtained from rats and mice but we detected no species effect of DR on the parameters we estimated. Due to a low number of experiments performed per single strain/genetic background, we could not statistically test differential responses across genetic backgrounds, which have been suggested to affect DR responses (Liao *et al.*, 2010; Swindell, 2012). Part of the heterogeneity we observed in the effect of DR is likely attributable to such effects.

In rats and mice, DR thus likely slows an irreversible cumulative process. This may involve a slower accumulation of somatic mutations or other damage, possibly mediated by oxidative stress

Table 1 Summary of the meta-analyses performed for the five parameters investigated. Average parameters, estimated via meta-analysis, of the controls are presented. Also reported are the change in hazard induced by DR, as estimated by the meta-analysis, with the associated P values, and this effect expressed as the increase in life expectancy in days

Model	Control	Hazard ratio of DR	P value of hazard ratio	Life extension via DR (days)
Gompertz				
ln(a)	−11.57	−0.25	0.27	33
ln(b)	−4.90	−0.23	<0.0001	173
Gompertz–Makeham				
ln(a)	−11.98	−0.12	0.63	14
ln(b)	−4.83	−0.23	<0.0001	166
m	5.71E-5	−8.57E-6	0.73	2

DR, dietary restriction.

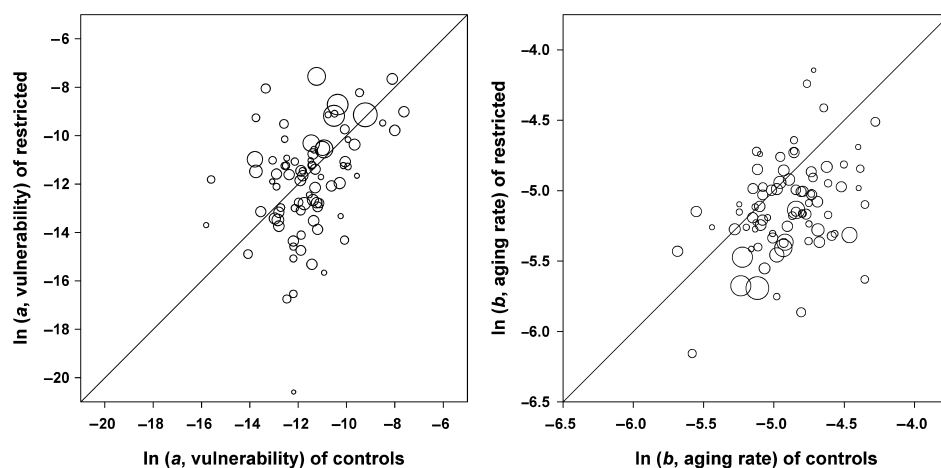


Fig. 1 Gompertz estimates obtained for the 82 pairs survival curves plotted as control against restricted, for *a* (left panel) and *b* (right panel). When points lie below the x equals y line, the parameter is lower under dietary restriction (DR). The bubble area represents the weight in the meta-analysis.

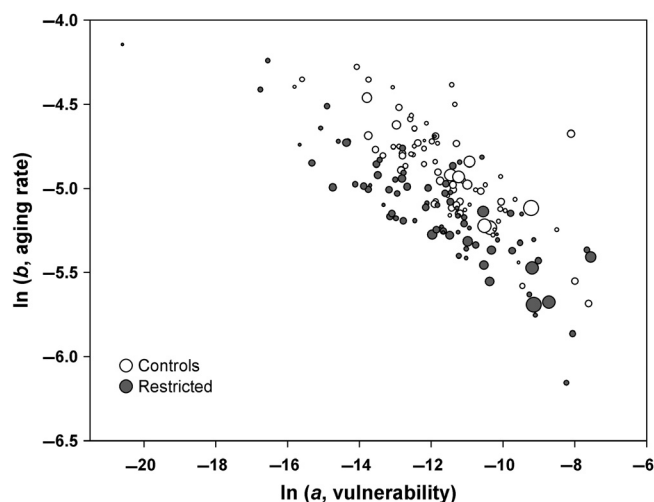


Fig. 2 Vulnerability $\ln(a)$ plotted against aging rate $\ln(b)$ (Gompertz model) of controls and dietary restriction (DR) to visualize the change in aging rate induced by DR without affecting vulnerability. The bubble area represents the weight in the meta-analysis.

(Gredilla & Barja, 2005). This implies that DR must be applied for a relatively long time to maximize the benefit from the associated lowered mortality risk. Additionally, it suggests that DR may be most effective when applied at times during life when damage accumulation is highest.

An apparent difference in aging rate may also arise when an intervention lowers mortality more successfully with increasing age. For example, the lifelong use of a walker may progressively reduce mortality risks with age, because falling risk accelerates with age. Thus, the possibility remains that our finding that aging rate is reduced by DR can be ascribed to DR negating mortality from a specific mortality cause of which the risk increases with age. Switching experiments (e.g. from control to DR and vice versa) can circumvent this confound, as were previously applied in *Drosophila* (Good & Tatar, 2001; Mair *et al.*, 2003). Studies switching between

control and DR have rarely been carried out in rodents, but see Forster *et al.* (2003) and Merry *et al.* (2008). However, the fact that we found that DR is less effective at old age in reducing aging rate suggests that the effect of DR on aging rate is real rather than apparent. Furthermore the few studies that initiated DR at old age in rodents reported, if anything, decreased life expectancy under DR (Lipman *et al.*, 1995; Forster *et al.*, 2003). Whether in other species than rodents in which DR extends lifespan (Nakagawa *et al.*, 2012), reduced vulnerability to the aging process does contribute, as in *Drosophila*, to the DR effect remains to be elucidated.

Interestingly, a study of the mortality trajectories of mice that were long- or short-lived due to genetic modification concluded that aging rate was affected in the minority of cases (de Magalhães, 2004; Yen *et al.*, 2008), especially in genetically modified mice with a long-lived phenotype (Yen *et al.*, 2008). The life-extending effect of Rapamycin may also arise via changed vulnerability to aging rather than slowed aging rate (Miller *et al.*, 2011). Thus, DR is perhaps the sole life-extending intervention that achieves this via slowing the rate of aging. Although short-term benefits of a lowered mortality rate via decreased vulnerability are appealing, slowing aging rate may still be the Holy Grail to extend life beyond certain limits. Lifespan gain via vulnerability likely reaches limits because irreversible damage accumulation continues at the same rate.

Experimental procedures

Data collection

Survival data from DR experiments in rats and mice were extracted from the literature. We searched both PubMed and Google Scholar and examined the reference lists of retrieved papers and reviews. Our inclusion criteria were as follows: (i) The experiment contained both a control group and a food restricted group. (ii) Survival was reported until all animals died and was extractable from tables or figures in at least five binned time intervals. (iii) Studies that used strains that were selected or genetically modified to be used as a

disease model or for short or long lifespan were excluded. (iv) The study had no possible confounding treatments, for example drugs or exercise. (v) We were careful not to include multiple publications of the same data set, as for example the data set of the National Institute of Aging (NIA)/National Center for Toxicological research (NCTR) cohorts (Turturro *et al.*, 1999), which can lead to multiple inclusion of the same data thereby biasing meta-analysis. (vi) Feeding schedules for the control and the restricted groups were similar, that is, we excluded experiments where animals were only fed every other day. (vii) From one study (Everitt *et al.*, 1982), we excluded one experimental group, because starvation leading to rapid mortality was observed. (viii) When multiple experimental groups with the same degree of DR were available (e.g. different timing of feeding) within the same study, we selected the experimental group of which the experimental protocol was most comparable to the control group.

Gompertz estimates

Data were directly measured from graphs [using ImageJ (Abramoff *et al.*, 2004)] or obtained from tables from the retrieved studies (Table S1). Estimates of mortality parameters are sensitive to substantial bias when linear regression on transformed data is applied to obtain them (Mueller *et al.*, 1995; Pletcher, 1999; Promislow *et al.*, 1999; Yen *et al.*, 2008). Maximum log-likelihood estimation (MLE) and nonlinear regression of survival curves (NLS) have been proposed as alternatives and they reduce this bias considerably (Pletcher, 1999; Promislow *et al.*, 1999; Pletcher *et al.*, 2000). We fitted both the Gompertz and the Gompertz–Makeham model (Eqn 1 and 2) with MLE (Survomatic) in R (Team, 2006), but also with NLS (in R), which is slightly more prone to bias in some cases. We arrived at the same conclusions using NLS as with MLE (data not shown). The Gompertz model corresponds to analyses performed by Nakagawa *et al.*, 2012, whereas the Gompertz–Makeham model may in some cases result in more accurate estimates of *a* and *b* (Golubev, 2004).

Gompertz hazard function

$$a \cdot e^{b \cdot t} \quad (1)$$

Gompertz–Makeham hazard function

$$m + a \cdot e^{b \cdot t} \quad (2)$$

Data synthesis

The parameters from the reanalyzed survival curves were summarized using meta-analysis with a random effects model fitted by restricted maximum likelihood [using the metafor package in R (Viechtbauer, 2010)]. To assess the effect of DR on either *a* or *b*, separate meta-analyses were performed using the ln of the ratio of these parameters (i.e. hazard ratio), the parameter under restriction was divided by the parameter under the control situation per combination of survival curves. For the Makeham parameter (*m*), we took the difference between DR and the control situation, because

this parameter was not log-distributed. Moderators were also tested as outlined in the results section.

In meta-analysis, studies are weighted by the inverse of the conditional variance of each study (Shadish & Haddock, 1994). Variance in the estimate of an effect decreases with increasing sample size. The relationship between conditional variance and sample size differs between different expressions of effect size (Shadish & Haddock, 1994). For a hazard ratio of Gompertz parameters, we do not know of a published relationship between conditional variance and sample size. Also within our set, the binning interval at which survival data are presented could decrease the confidence in the parameters estimated. We therefore used simulations to estimate the relationship between conditional variance of the hazard ratio of the Gompertz and Gompertz–Makeham parameters with sample size and with binning interval. Using fixed Gompertz and Gompertz–Makeham hazard functions (Eqn 1 and 2), individual lifespan data were generated (in R) by drawing from a uniform distribution (between 0 and 1) for each individual at each successive time point until the drawn number was lower than the hazard determined by the hazard function resulting in a simulated death.

The effect of binning interval on conditional variance was minor compared with the effect of sample size (*N*), but was apparent at large binning intervals (i.e. a low total number of bins). Given that binning interval had little effect on the confidence of the hazard ratio of the parameters, we estimated the conditional variance with sample size alone, which showed an inverse relationship with sample size (closely following a $x \cdot N^2$ function). Because the hazard ratios of the Gompertz parameters are not standardized metrics of effect size, conditional variance depends on the size of the underlying Gompertz parameters. Therefore, we estimated 'x' and 'z' in $x \cdot N^2$ using simulations with the mean values of the parameters obtained from the published survival curves. In some studies, a control group was used in multiple comparisons; in these cases, the total *N* was adjusted by dividing it by the times it was used.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Fig. S1 Gompertz–Makeham model results, refer to legend of figure 1 in manuscript for description.

Fig. S2 Gompertz–Makeham model results, refer to legend of figure 2 in manuscript for description.

Table S1 Data collected including references to literature used.

Dialog S1 Figures showing the bias inherent in the linear regression approach.